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5/15/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES
(Case No. 99-499)

In the Application of:

Chou et al.

Serial No.: 08/903,944

Filing Date: July 31, 1997

For: Production of Transgenic Poinsettia

Examiner: D. Fox

Group Art Unit: 1638

TRANSMITTAL LETTER

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In regard to the above identified application,

1. We are transmitting herewith the attached:

   a) Appeal Brief with Appendix A (in triplicate); and
   b) return receipt postcard.

2. With respect to fees:

   a) Please charge our Deposit Account, No. 13-2490, in the amount of $320.00.
   b) Please charge any underpayment or credit any overpayment our Deposit Account, No. 13-2490.

3. CERTIFICATE OF MAILING UNDER 37 CFR § 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described in paragraph 1, are being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Commissioner for Patents, Washington, D.C. 20231 on April 23, 2002.

Respectfully submitted,

Date: April 23, 2002

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BRIEF ON APPEAL

Commissioner for Patents
Washington, D.C. 20231

Sir:

The applicants hereby file an original and three copies of this appeal brief.

I. REAL PARTY IN INTEREST

The real party in interest is The Scotts Company of Marysville, Ohio.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals and interferences.

III. STATUS OF CLAIMS

Upon filing of this appeal and entry of the applicants' after final response:

Claims 1–3, 6–45, 47–106, and 108–118 are pending and rejected, which rejections are appealed from.

Claims 4 and 5 are pending and objected to as depending from a rejected claim.

A clean set of the claims as pending upon entry of the after-final response is attached as Appendix A.

IV. STATUS OF AMENDMENTS

The Advisory Action mailed November 29, 2001 (Paper No. 32) stated that the amendments filed on November 9, 2001, would be entered.

V. SUMMARY OF THE INVENTION

The presently claimed invention comprises methods of regenerating poinsettia *in vitro* and methods of producing transgenic poinsettia plants. Poinsettia plants are the primary potted flowering plant produced and sold in North America. P. 1, lns. 23-25. Accordingly, improved methods of regenerating poinsettia *in vitro* are desirable and provided by the presently claimed invention.

Poinsettia, like many plants, are susceptible to a number of insect pests and diseases, even under greenhouse conditions. P. 2, lns. 5-6. While chemical treatment can control certain pests and disease-causing pathogens, they can also have deleterious effects on the plants. P. 2, lns. 25-27. An alternative to chemical treatment is to genetically engineer poinsettia that express polypeptides capable of protecting the plant against insects and pathogens as well as to enhance the commercial value of the poinsettia by controlling various commercially valuable phenotypes. P. 2, lns. 27-36. Until the filing of the present application, no transgenic poinsettia have been produced. P. 3, lns. 1-2.

The present claims provide for the first time methods of producing transgenic plants and the plants thereby produced. P. 3, lns. 7-21, p. 9, lns. 31-33. Such transgenic poinsettia plants can be made to express proteins that assist the plant in combating pests and disease. P. 5, lns. 29-33.

The method of *in vitro* regeneration generally comprises:

(a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on callus induction medium;

(b) subculturing reddish epidermal callus to embryo induction medium to form embryogenic callus;

(c) culturing said embryogenic callus on developmental medium;

(d) culturing said embryogenic callus on maturation medium; and

(e) recovering poinsettia plants from said embryos.

P. 3, lns. 23-32.

The method of producing transgenic poinsettia plants generally comprises:

(a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on a callus induction medium;

(b) culturing reddish epidermal callus on embryo induction medium to form embryogenic callus;

(c) introducing one or two expression vectors containing selectable marker genes into said incubating embryogenic callus by *Agrobacterium tumefaciens* mediated transformation or by microprojectile-mediated delivery of the vectors into the callus to produce transformed embryogenic callus;

(d) culturing said transformed embryogenic callus on selection medium;

(e) culturing said transformed embryogenic callus containing embryos on developmental medium;

(f) culturing said transgenic embryos on maturation medium; and

(g) recovering transgenic plants from said transgenic embryos.

P. 3, ln. 33, to p. 4, ln. 20. A wide variety of selectable marker genes (*e.g.*, p. 12, lns. 17, to p. 13, ln. 11), and the expression vectors can comprise a wide variety of genes whose expression products permit the transgenic plant to combat infections by pest and other pathogens (p. 28, ln. 25 *et seq.*) as well as cause the transgenic plant to exhibit aesthetically pleasing phenotypes (p. 38, ln. 37 *et seq.*).

VI. ISSUES

The issues presented to the Board after entry of the after-final amendment are the following:

A. Whether claims 6–37, 39–45,47–71, 73–96, 98–100, 102–103, 105–106, 108–112, 114–115, and 117-118 are non-enabled under 35 U.S.C. § 112, first paragraph, for methods of transformation using *Agrobacterium tumefaciens*.

B. Whether claims 1–3, 97, 101, 104, 113, and 116 are obvious under 35 U.S.C. § 103(a) over Preil taken with Nataraja in light of Lee *et al.*

C. Whether claims 1–3, 6–8, 11–41, 44–45, 47–106, and 108–118 are obvious under 35 U.S.C. § 103(a) over Miki taken with Preil (1994) and Nataraja in light of Lee *et al.*

VII. GROUPING OF THE CLAIMS

A. With regard to the rejection under 35 U.S.C. § 112, firs paragraph, as non-enabled for methods of transformation using *Agrobacterium tumefaciens*, all the claims rejected for this reason stand or fall together (*i.e.*, claims 6–37, 39–45,-47–71, 73–96, 98–100, 102–103, 105–106, 108–112, 114–115, and 117-118).

B. With regard to the rejection under 35 U.S.C. § 103(a) over Preil (in "The Scientific Basis of Poinsettia Production," Stromme, Ed., pages 49-56, The Agricultural University of Norway 1994) taken with Nataraja (*Current Science* **42**, 577 (1973)) in light of Lee (*Nature Biotech.* **15**, 178-182 (1997)), all the claims rejected for this reason stand or fall together (*i.e.*, claims 1–3, 97, 101, 104, 113, and 116).

C. With regard to the rejection under 35 U.S.C. § 103(a) over Miki (in "Methods in Plant Molecular Biology and Biotechnology," Glick *et al.*, Eds., pp. 67-88, CRC Press 1993) taken with Preil and Nataraja in light of Lee *et al.*, all of the rejected claims stand or fall together (*i.e.*, claims 1–3, 6–8, 11–41, 44–45, 47–106, and 108–118).

VIII. ARGUMENT

A. Rejection under 35 U.S.C. § 112, first paragraph

The claims are rejected as non-enabled for *Agrobacterium tumefaciens*-mediated transformation. The Examiner alleged that the transformation of poinsettia resulting in whole transformed plants is unpredictable as evidenced by Follansbee *et al.* (*In Vitro* **31**(3), p. 72A (1999), which demonstrated that whole *Euphorbia* (poinsettia) cannot be recovered following *A. rhizogenes* transformation. The applicants respectfully traverse.

As noted, Follansbee used *Agrobacterium rhizogenes* and not *Agrobacterium tumefaciens* as recited in the appealed claims. The difference is significant because *Agrobacterium rhizogenes* systems are designed for obtaining transgenic roots, not whole plants. *Agrobacterium tumefaciens* systems, on the other hand, have been developed to obtain whole plants, such as presently disclosed and claimed. Those skilled in the art would not consider Follansbee *et al.*'s work with *Agrobacterium rhizogenes* indicative or predictive of the likely outcome with *Agrobacterium tumefaciens* systems.

The Examiner alleged that *A. rhizogenes*, as taught by Follansbee *et al.*, may be manipulated to delete root-inducing genes as taught by Miki at page 67-71. Apparently, the Examiner is suggesting

that *A. rhizogenes* could be manipulated to become the same or similar to *A. tumefaciens* and, therefore, Follansbee *et al.*'s suggestion that *A. rhizogenes* does not successfully transform whole *Euphorbia* plants would apply equally to *A. tumefaciens*. But Miki does not teach or suggest manipulation of *A. rhizogenes* to delete the root-inducing genes, and neither Follansbee nor Miki disclose the result of transformation of poinsettia using *A. rhizogenes* having deleted root-inducing genes or using *A. tumefaciens*. The Examiner merely speculates that *A. rhizogenes* or *A. rhizogenes* having deleted root-inducing genes would react the same way as *A. tumefaciens*. There is no support for such an assumption in the scientific literature and none in the record.

Because the teachings of Follansbee are not relevant to the *Agrobacterium tumefaciens* system, there remains no evidence or scientific reasoning of record that suggests that the *Agrobacterium tumefaciens* system would not function as disclosed and claimed. Without such support, this enablement rejection cannot be sustained. *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971) ("[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.").

In view of the foregoing, the applicants respectfully request reversal of this rejection.

B. Rejection under 35 U.S.C. § 103 over Preil taken with Nataraja in light of Lee *et al.*

Claims 1–3, 97, 101, 104, 113, and 116 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Preil taken with Nataraja in light of Lee For the following reasons, the applicants respectfully traverse this rejection.

1. The cited art fails to teach or suggest all the elements recited in the pending claims

The prior art fails to teach or suggest the particular multi-step processes for poinsettia regeneration, with or without prior transfection with an expression vector. For example, claim 1 recites a method comprising:

(a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) subculturing reddish epidermal callus to embryo induction medium comprising casein hydrolysate and NH_4^+ and/or NO_3^- to form embryogenic callus;

(c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and cytokinin;

(d) culturing said embryogenic callus on maturation medium comprising abscisic acid; and

(e) recovering poinsettia plants from said embryos.

Each of the claimed methods of producing transgenic poinsettia's also recite these actions.

i. The cited art fails to teach using both a developmental medium and a maturation medium

By contrast, the primary reference relied upon for this rejection, Priel *et al.*, teaches at p. 50, 1st column that placing stem segments on "callus induction medium" followed by transfer to "somatic embryogenesis induction medium" where, after transfer to fresh medium, the first embryogenic structures become visible. The embryogenic structures are then placed on "somatic embryo maturation medium," where they reach the cotyledonary stage.

But neither Priel nor the other cited art teach or suggest placing the embryogenic callus on both a developmental medium and a maturation medium; Priel teaches only placing the embryogenic structures on a maturation medium. Thus, even were one to combine the teachings of the prior art, one would not arrive at the instantly claimed methods. And there is not even a specific allegation that using both a developmental medium and a maturation medium would have been obvious to one of ordinary skill in the art.

Furthermore, the present specification notes at page 16, lines 7-9, that "[t]his additional treatment improves embryo germination uniformity and confers a high degree of desiccation tolerance." This inherent advantage in the presently claimed methods is neither taught or suggested by the cited art.

ii. The cited art fails to teach the use of casein hydrolysate in somatic embryogenesis

The Examiner has relied on Nataraja for the use of casein hydrolysate in embryo culture medium for poinsettia. The Examiner correctly notes Nataraja *et al.*'s use was for zygotic rather than somatic

embryos, but concludes that one skilled in the art would have recognized that it could be used equally well with each type of embryo. No evidence in support of this assertion has been provided.

The Examiner maintains that the use of casein hydrolysate as a tissue culture medium addition is well known, yet no teaching or suggestion has been provided for its use in poinsettia somatic embryogenesis.

Nataraja teaches the use of casein hydrolysate in a medium for culturing de-coated seeds, which enlarged with the subsequent emergence of radicle that swelled with proliferation of the hypocotyls region to yield a mass of yellowish-brown, fleshy callus. By contrast, the presently claimed invention employs casein hydrolysate in an embryo induction medium to subculture reddish epidermal callus to form embryogenic callus.

Furthermore, casein hydrolysate is but a single compound among many employed by Nataraja, and Nataraja does not distinguish it from any of the other compounds as being particularly desirable for use in *in vitro* culturing of poinsettia. There is simply no motivation provided in the cited art to pluck this particular compound from among the many disclosed by Nataraja for use in the method of Priel and as recited in the present claims. (See also the argument in VIII.B.2 noting that prior art must suggest particular elements for combination.)

Lastly, Preil was published in 1994, *20 years* after the publication of Nataraja, and provides a review of procedures for *in vitro* culturing of poinsettia available at the time. Presumably Preil *et al.*, as with any such review, disclosed the best methods known at the time. Surely had it been desirable to employ casein hydrolysate as taught by Nataraja in 1974, Preil would have incorporated such a teaching in its review 20 years later. Yet such a teaching is conspicuously absent.

iii. The cited art fails to teach other elements of the claimed methods

As to the other media components, the Examiner alleges they are well known. Yet no evidence of this assertion has been made of record, as required to support this rejection. *In re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002) (obviousness rejections must be based on objective evidence of record). For example, "the Examiner maintains that the use of the particularly claimed nitrogen sources in tissue

culture media is well known, as is the use of abscisic acid." Yet, no evidence in support of this allegation is of record.

More to the point, however, is that the claims recite NH_4^+ and/or NO_3^- in the embryo induction medium and abscisic acid an a maturation medium, not these components in a generic "tissue culture" media as asserted by the Examiner. Neither Preil nor Nataraja contain such a teachings or suggestions.

Nor do these references teach an osmotic pressure increasing agent in a developmental medium. The Examiner merely alleges that "any carbohydrate or salt will change the osmotic pressure "to some degree" without any prior art support.

2. *The prior art fails to teach or suggest the particular combination of elements now being claimed*

The applicants respectfully submit that the Examiner has relied on particular teachings that were selectively culled from the prior art without any suggestion in the references themselves or reason why such selections would have distinguished themselves to those of ordinary skill in the art. Obviousness cannot be predicated on the mere identification of elements in the prior art – a clear identification as to the reason one of ordinary skill in the art would have selected these elements must be made. *In re Kotzab*, 55 USPQ2d 1313 (Fed. Cir. 2000).

There is simply no teaching or suggestion to make the various *combination* of modifications to prior art methods of *in vitro* regeneration of poinsettia as presently claimed. One cannot establish a *prima facie* case of obviousness without identifying in the art suggestion or motivation to make the *particular* invention being claimed. *In re Deuel*, 51 F.3d 1552, 1559 (Fed. Cir. 1995) ("A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out."); and *Ex parte Obukowicz*, 27 U.S.P.Q.2d, 1063, 1065 (Bd. Pat. App. Int. 1992) (Prior art "that gives only general guidance and is not at all specific as to the particular form of the claimed invention and how to achieve it . . . does not make the invention obvious.").

3. *The cited art fails to imbue the ordinary artisan with a reasonable expectation of success*

In the July 7, 1999, Office Action, the Examiner noted, "[Obtaining] whole poinsettia plants from tissue culture is unpredictable, given the highly genotype-dependent techniques available at the time of the invention and the recalcitrance of transformed *Euphorbia* cells to produce whole plants." Given this unpredictability, there could not have been a reasonable expectation of successfully making and using the presently claimed method.

Furthermore, the prior art includes examples of failures of others. In the September 25, 1998, Office Action, the Examiner applied Cheetham against the claims in an obviousness rejection. Cheetham attempted to regenerate shoots from cultured root explants of poinsettia, but they never succeeded (the publication expressly stating on p. 513 that "no shooting was ever observed." Furthermore, the applicants submitted correspondence between one of the inventors and Dr. P. Weathers, one of the authors of the Cheetham article in which Dr. Weathers states, "We tried various regimens of hormones to get plant regeneration . . . . No shoots were ever observed. The applicants subsequently submitted the biography of Dr. Weathers, which identified her as being of at least ordinary skill in the art. Given the failure of others, one of ordinary skill in the art could not have had a reasonable expectation of success.

Without a reasonable expectation of success, the claim invention cannot be obvious. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

4. *The cited art fails to teach or suggest the inherent advantages of the claimed method*

There is nothing in the prior art that suggests to the ordinary artisan the other inherent advantages of the presently claimed methods. For example, page 15 of the present specification states that the presently claimed methods are genotype-independent vis-à-vis producing large quantities of somatic embryos of improved quality and yield. Such inherent properties of the present methods were not recognized in the art.

The Examiner dismissed the applicants' argument that the present methods were genotype-independent, arguing that such methods rely upon high levels of a particular osmoticum and high lev-

els of particular nitrogen source. The Examiner has provided no basis for such an assertion, however.

For all of the foregoing reasons, the applicants respectfully request that this § 103 rejection be withdrawn.

C. Rejection of under 35 U.S.C. § 103 over Miki taken with Preil and Nataraja in light of Lee *et al.*

Claims 1–3, 6–8, 11–41, 44–45, 47–106, and 108–118 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Miki taken with Preil and Nataraja in light of Lee For the following reasons, the applicants respectfully traverse this rejection.

This rejection is substantively the same as the prior obviousness rejection with the exception that Miki is further relied upon for its teaching of particle bombardment technique for plant transformation. But Miki fails to compensate for the deficiencies of the combination of Preil, Nataraja, and Lee noted above, and, therefore, for the very same reasons presented above, claims 1–3, 6–8, 11–41, 44–45, 47–106, and 108–118 cannot be obvious over the combination of Miki taken with Preil and Nataraja in light of Lee For all the reasons presented, the applicants respectfully request that this § 103 rejection be withdrawn.

Respectfully submitted,

Date: April 23, 2002

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APPENDIX A

Claims of Application Serial No. 08/903,944

1. (Three Times Amended) A method for in vitro regeneration of poinsettia plants comprising:
   (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
   (b) subculturing reddish epidermal callus to embryo induction medium comprising casein hydrolysate and NH_4^+ and/or NO_3^- to form embryogenic callus;
   (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and cytokinin;
   (d) culturing said embryogenic callus on maturation medium comprising abscisic acid; and
   (e) recovering poinsettia plants from said embryos.

2. The method of claim 1, wherein said callus induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine 400 to 1700mb/liter NH_4NO_3, 1900 to 3500 mb/liter KNO_3 and about 1 gm liter casein hydrosylate.

3. The method of claim 1, wherein said embryo induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine 400 to 1700mb/liter NH_4NO_3, 1900 to 3500 mb/liter KNO_3 and about 1 gm liter casein hydrosylate.

4. The method of claim 1, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

5. The method of claim 1, wherein said maturation medium comprises about 5-20 µM abscisic acid, about 30-100gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

6. (Four Times Amended) A method for producing transgenic poinsettia plants, comprising:
   (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and NH_4^+ and/or NO_3^- to form embryogenic callus;

(c)

(i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with Agrobacterium tumefaciens containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

(d) culturing said transformed embryogenic callus on selection medium;

(e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(f) culturing said transgenic embryos on maturation medium; and

(g) recovering transgenic plants from said transgenic embryos.

7. The method of claim 6, wherein said callus induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm liter casein hydrosylate.

8. The method of claim 6, wherein said embryo induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm liter casein hydrosylate.

9. The method of claim 6, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

10. The method of claim 6, wherein said maturation medium comprises about 5-20 μM abscisic acid, about 30-100gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

11. The method of claim 8 wherein said embryo induction medium further comprises about 400 to 1700mg/liter NH_4NO_3, 1900 to 3500 mg/liter KNO_3.

12. The method of claim 6, wherein said poinsettia plant tissue explants are selected from the group consisting of immature embryos, mature embryos, shoot tips and stem segments.

13. The method of claim 6, wherein said selectable marker gene is selected from the group consisting of a neomycin phosphotransferase gene, a hygromycin phosphotransferase gene, a phosphinothricin gene, a dihydrofolate reductase gene, a 5-enolpyruvylshikimate-3-phosphate synthase gene, an acetohydroxyacid synthase gene, a chloramphenicol acetyltransferase gene, a 3"-adenylyltransferase gene, a gentamicin acetyltransferase gene, a streptomycin phosphotransferase gene, and an aminoglycoside-3'-adenyl transferase gene.

14. The method of claim 13, wherein said selectable marker gene in hygromycin phosphotransferase and said selection agent is hygromycin.

15. The method of claim 6, wherein said expression vector that comprises said second foreign gene further comprises a promoter, wherein said promoter is selected from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, wherein the expression of said second foreign gene is under the control of said promoter.

16. The method of claim 15, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

17. (Amended) The method of claim 6, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus.

18. (Twice Amended) The method of claim 17, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2′-5′ oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

19. (Amended) The method of claim 6, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein selected from the group consisting of tryptophan decarboxylase, lectin, and Bacillus thuringiensis toxin.

20. The method of claim 19 wherein said lectin is Galanthus nivalis lectin.

21. The method of claim 6, wherein said second foreign gene confers resistance to a bacterium or a fungus, and wherein said second foreign gene encodes a polypeptide selected from the group consisting of chitinase, a β-1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

22. The method of claim 21, wherein said plant defensin is radish seed Rs-AFP2.

23. The method of claim 21, wherein said lytic peptide is selected from the group consisting of a magainin, PGLa, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

24. The method of claim 6, wherein said second foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

25. The method of claim 23, wherein said magainin is magainin 1 or magainin 2.

26. The method of claim 6, wherein said transgenic poinsettia comprises an expression vector that further comprises a third foreign gene.

27. The method of claim 6, wherein said second foreign gene encodes chitinase, and wherein said third foreign gene encodes β-1,3-glucanase.

28. The method of claim 6, wherein said second foreign gene encodes magainin 2, and wherein said third foreign gene encodes PGLa or PGL.

29. The method of claim 6, wherein the expression of said second foreign gene confers insensitivity to ethylene, and wherein said second foreign gene encodes a mutated ethylene receptor.

30. The method of claim 29, wherein said mutated ethylene receptor gene is the Arabidopsis etr-1 gene or a tomato NR gene.

31. The method of claim 6, wherein said second foreign gene is the Vitreoscilla hemoglobin gene.

32. The method of claim 6, wherein said second foreign gene is an isopentyenyl transferase gene, wherein the expression of said isopentynyl transferase gene is under the control of a promoter of a senescence-associated gene.

33. The method of claim 32, wherein said promoter is the Arabidopsis SAG12 gene promoter.

34. The method of claim 6, wherein said second foreign gene encodes a polypeptide having a MADS box domain.

35. The method of claim 34, wherein said second foreign gene is selected from the group consisting of the PLENA gene, the SQUAMOSA gene, the DEFICIENS A gene, the GLOBOSA gene, the APTELA1 gene, the APETALA2 gene, the AGAMOUS gene, the OsMADS24 gene, the OsMADS45 gene, and the OsMADS1 gene.

36. The method of claim 6, wherein said foreign gene encodes a protein that modifies plant habit.

37. The method of claim 36, wherein said gene is the OsMADS1 or phyA gene.

38. The method of claim 6, wherein said expression vector is introduced by microparticle bombardment.

39. (Five Times Amended) A method for producing transgenic poinsettia plants, comprising:

(a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;

(b) subculturing embryogenic callus produced on said callus induction medium to liquid NH_4^+ and/or NO_3^- containing embryo induction medium;

(c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;

(d) filtering the culture and culturing the filtrate on solid embryo induction medium;

(e) subculturing embryos produced on said embryo induction medium to maturation medium;

(f) culturing said embryos on callus induction medium;

(g) subculturing epidermal callus produced on said callus induction medium to embryo induction medium to form embryogenic callus;

(h)

(i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with Agrobacterium tumefaciens containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

(i) culturing said transformed embryogenic callus on selection medium;

(j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(k) culturing said transformed embryos on maturation medium; and

(l) recovering transgenic plants from said transgenic embryos.

40. The method of claim 39, wherein said callus induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm liter casein hydrosylate.

41. The method of claim 39, wherein said embryo induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm liter casein hydrosylate.

42. The method of claim 39, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

43. The method of claim 39, wherein said maturation medium comprises about 5-20 μM abscisic acid, about 30-100gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

44. The method of claim 40 wherein said embryo induction medium further comprises about 400 to 1700mg/liter NH_4NO_3, 1900 to 3500 mg/liter KNO_3.

45. The method of claim 39, wherein said poinsettia plant tissue explants are selected from the group consisting of immature embryos, mature embryos, shoot tips and stem segments.

47. The method of claim 39, wherein said selectable marker gene is selected from the group consisting of a neomycin phosphotransferase gene, a hygromycin phosphotransferase gene, a phosphinothricin gene, a dihydrofolate reductase gene, a 5-enolpyruvylshikimate-3-phosphate synthase gene, an acetohydroxyacid synthase gene, a chloramphenicol acetyl-transferase gene, a 3′-adenylyltransferase gene, a gentamicin acetyltransferase gene, a streptomycin phosphotransferase gene, and an aminoglycoside-3'-adenyl transferase gene.

48. The method of claim 47, wherein said selectable marker gene is hygromycin phosphotransferase and said selection agent is hygromycin.

49. The method of claim 47, wherein said expression vector that comprises said second foreign gene further comprises a promoter, wherein said promoter is selected from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, wherein the expression of said second foreign gene is under the control of said promoter.

50. The method of claim 49, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

51. (Amended) The method of claim 39, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus.

52. (Twice Amended) The method of claim 51, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2′-5′ oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

53. (Twice Amended) The method of claim 39, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein selected from the group consisting of tryptophan decarboxylase, lectin, and Bacillus thuringiensis toxin.

54. The method of claim 53 wherein said lectin is Galanthus nivalis lectin.

55. The method of claim 39, wherein said second foreign gene confers resistance to a bacterium or a fungus, and wherein said second foreign gene encodes a polypeptide selected from the group consisting of chitinase, a β-1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

56. The method of claim 55, wherein said plant defensin is radish seed Rs-AFP2.

57. The method of claim 55, wherein said lytic peptide is selected from the group consisting of a magainin, PGLa, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

58. The method of claim 39, wherein said second foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

59. The method of claim 39, wherein said magainin is magainin 1 or magainin 2.

60. The method of claim 39, wherein said transgenic poinsettia comprises an expression vector that further comprises a third foreign gene.

61. The method of claim 39, wherein said second foreign gene encodes chitinase, and wherein said third foreign gene encodes β-1,3-glucanase.

62. The method of claim 39, wherein said second foreign gene encodes magainin 2, and wherein said third foreign gene encodes PGLa or PGL.

63. The method of claim 39, wherein the expression of said second foreign gene confers insensitivity to ethylene, and wherein said second foreign gene encodes a mutated ethylene receptor.

64. The method of claim 63, wherein said mutated ethylene receptor gene is the Arabidopsis etr-1 gene or a tomato NR gene.

65. The method of claim 39, wherein said second foreign gene is the Vitreoscilla hemoglobin gene.

66. The method of claim 39, wherein said second foreign gene is an isopentyenyl transferase gene, wherein the expression of said isopentynyl transferase gene is under the control of a promoter of a senescence-associated gene.

67. The method of claim 66, wherein said promoter is the Arabidopsis SAG12 gene promoter.

68. The method of claim 39, wherein said second foreign gene encodes a polypeptide having a MADS box domain.

69. The method of claim 68, wherein said second foreign gene is selected from the group consisting of the PLENA gene, the SQUAMOSA gene, the DEFICIENS A gene, the GLOBOSA gene, the APTELA1 gene, the APETALA2 gene, the AGAMOUS gene, the OsMADS24 gene, the OsMADS45 gene, and the OsMADS1 gene.

70. The method of claim 39, wherein said foreign gene encodes a protein that modifies plant habit.

71. The method of claim 70, wherein said gene is the OsMADS1 or phyA gene.

72. The method of claim 39, wherein said expression vector is introduced by microparticle bombardment.

73. A transgenic poinsettia plant comprising at least one expression vector, wherein said expression vector comprises at least one foreign gene, and wherein said transgenic poinsettia plant expresses said foreign gene.

74. The transgenic poinsettia plant of claim 73, wherein said expression vector further comprises a promoter, wherein said promoter is selected from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, wherein the expression of said foreign gene is under the control of said promoter.

75. The transgenic poinsettia plant of claim 74, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

76. (Twice Amended) The transgenic poinsettia plant of claim 73, wherein the expression of said foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus.

77. (Twice Amended) The transgenic poinsettia plant of claim 76, wherein said foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2′-5′ oligonucleotide synthetase, viral genome antisense RNA, and pokeweed B81antiviral protein.

78. (Twice Amended) The transgenic poinsettia plant of claim 73, wherein said foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein selected from the group consisting of tryptophan decarboxylase, lectin, and Bacillus thuringiensis toxin.

79. The transgenic poinsettia plant of claim 78 wherein said lectin is Galanthus nivalis lectin.

80. The transgenic poinsettia plant of claim 76, wherein said foreign gene confers resistance to a bacterium or a fungus, and wherein said second foreign gene encodes a polypeptide selected from the group consisting of chitinase, a β-1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

81. The transgenic poinsettia plant of claim 80, wherein said plant defensin is radish seed Rs-AFP2.

82. The transgenic poinsettia plant of claim 80, wherein said lytic peptide is selected from the group consisting of a magainin, PGLa, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

83. The transgenic poinsettia plant of claim 73, wherein said foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

84. The transgenic poinsettia plant of claim 82, wherein said magainin in magainin 1 or magainin 2.

85. The transgenic poinsettia plant of claim 73, wherein said transgenic poinsettia comprises an expression vector that further comprises a second foreign gene.

86. The transgenic poinsettia plant of claim 85, wherein said foreign gene encodes chitinase, and wherein said second foreign gene encodes β-1,3-glucanase.

87. The transgenic poinsettia plant of claim 86, wherein said foreign gene encodes magainin 2, and wherein said second foreign gene encodes PGLa or PGL.

88. The transgenic poinsettia plant of claim 86, wherein the expression of said foreign gene confers insensitivity to ethylene, and wherein said foreign gene encodes a mutated ethylene receptor.

89. The transgenic poinsettia plant of claim 88, wherein said mutated ethylene receptor gene is the Arabidopsis etr-1 gene or a tomato NR gene.

90. The transgenic poinsettia plant of claim 73, wherein said foreign gene is the Vitreoscilla hemoglobin gene.

91. The transgenic poinsettia plant of claim 73, wherein said foreign gene is an isopentyenyl transferase gene, wherein the expression of said isopentynyl transferase gene is under the control of a promoter of a senescence-associated gene.

92. The transgenic poinsettia plant of claim 91, wherein said promoter is the Arabidopsis SAG12 gene promoter.

93. The transgenic poinsettia plant of claim 73, wherein said foreign gene encodes a polypeptide having a MADS box domain.

94. The transgenic poinsettia plant of claim 93, wherein said second foreign gene is selected from the group consisting of the PLENA gene, the SQUAMOSA gene, the DEFICIENS A gene, the GLOBOSA gene, the APTELA1 gene, the APETALA2 gene, the AGAMOUS gene, the OsMADS24 gene, the OsMADS45 gene, and the OsMADS1 gene.

95. The transgenic poinsettia plant of claim 73, wherein said foreign gene encodes a protein that modifies plant habit.

96. The transgenic poinsettia plant of claim 95, wherein said gene is the OsMADS1 or phyA gene.

97. The method of claim 1, wherein said poinsettia plants of step (e) are fertile.

98. The method of claim 6, wherein said poinsettia plants of step (g) are fertile.

99. The method of claim 39, wherein said poinsettia plants of step (l) are fertile.

100. The transgenic poinsettia plant of claim 73, wherein said plant is fertile.

101. (Twice Amended) A method for in vitro regeneration of poinsettia plants comprising:

(a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) subculturing reddish epidermal callus to NH_4^+ and/or NO_3^- containing embryo induction medium to form embryogenic callus containing embryos;

(c) culturing said embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent and cytokinin;

(d) culturing said embryogenic callus containing embryos on maturation medium comprising abscisic acid; and

(e) recovering poinsettia plants from said embryos.

102. (Three Times Amended) A method for producing transgenic poinsettia plants comprising the steps of:

(a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) subculturing embryogenic callus to embryo induction medium comprising casein hydrolysate and NH_4^+ and/or NO_3^- to form embryogenic callus containing embryos;

(c)

(i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with Agrobacterium tumefaciens containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

(d) culturing said transformed embryogenic callus on selection medium;

(e) culturing said embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(f) culturing said transgenic embryos on maturation medium; and

(g) recovering transgenic plants from said transgenic embryos.

103. (Twice Amended) A method for producing transgenic poinsettia plants comprising the steps of:

(a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) subculturing embryogenic callus produced on said callus induction medium to liquid embryo induction medium comprising casein hydrolysate and NH_4^+ and/or NO_3^-;

(c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;

(d) filtering the culture and culturing the filtrate on solid embryo induction medium;

(e) subculturing embryos produced on said embryo induction medium to maturation medium;

(f) culturing said embryos on callus induction medium;

(g) subculturing embryogenic callus produced on said callus induction medium to embryo induction medium to form embryogenic callus containing embryos;

(h)

(i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with Agrobacterium tumefaciens containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

(i) culturing said transformed embryogenic callus on selection medium;

(j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(k) culturing said transformed embryos on maturation medium; and

(l) recovering transgenic plants from said transgenic embryos.

104. The method of claim 101, wherein said developmental medium comprises about 0.05 mg/liter cytokinin.

105. The method of claim 102, wherein said developmental medium comprises about 0.05 mg/liter cytokinin.

106. The method of claim 103, wherein said developmental medium comprises about 0.05 mg/liter cytokinin.

108. The method of claim 102, wherein said developmental medium comprises cytokinin.

109. The method of claim 103, wherein said developmental medium comprises cytokinin.

110. The method of claim 6, wherein the expression of said second foreign gene confers resistance to an insect.

111. (Amended) The method of claim 39, wherein the expression of said second foreign gene confers resistance to an insect.

112. The transgenic poinsettia plant of claim 73, wherein the expression of said second foreign gene confers resistance to an insect.

113. The method according to claim 1, wherein the nitrogen source comprises NH_4^+ and/or NO_3^-.

114. The method according to claim 6, wherein the nitrogen source comprises NH_4^+ and/or NO_3^-.

115. The method according to claim 39, wherein the nitrogen source comprises NH_4^+ and/or NO_3^-.

116. The method according to claim 101, wherein the nitrogen source comprises NH_4^+ and/or NO_3^-.

117. The method according to claim 102, wherein the nitrogen source comprises NH_4^+ and/or NO_3^-.

118. The method according to claim 103, wherein the nitrogen source comprises casein hydrolysate and NH_4^+ and/or NO_3^-.